REMARKS

Claims 1 and 23 are amended to claim *ex vivo* methods. Support for the amendments appears in at least Example 6. Claims 1-26 are pending, and claim 22 is withdrawn from consideration.

I. 35 U.S.C. § 112, second paragraph

Claim 4 stands rejected under 35 U.S.C. §112, second paragraph as indefinite how a "double stranded nucleic acid" can interact by Watson-Crick base pairing to the adaptor nucleic acid when the double stranded nucleic acid has no single stranded regions.

Applicants respectfully traverse this rejection.

Claim 4 is clear and definite to one skilled in the art. According to claim 4, a double stranded donor nucleic acid can interact with the adapter segment via Watson-Crick interaction by invasion of the double stranded donor nucleic acid by the adapter segment ("strand invasion"). For instance, strand invasion with peptide nucleic acids ("PNA") is described in the literature. Wang and Xu, "*Peptide nucleic acid (PNA) binding mediated gene regulation*", 14 (2) CELL RESEARCH 111-116 (2004) state on page 111, left column (emphasis added):

> PNAs can bind to both DNA and RNA targets in a sequence-specific manner to form Watson-Crick type PNA/DNA and PNA/RNA double helical structure. PNAs can also bind to double-stranded DNA (ds DNA) targets. In this case the PNA molecule replaces one of the complimentary DNA strands by strand then exists as a single-stranded D-loop at the PNA binding site.

The specification of the instant application supports such Watson-Crick interaction by strand invasion. For example, the specification describes that oligonucleotides according to the invention, *e.g.*, the adapter segment, may comprise modified oligonucleotide backbones such as PNA (see specification [0088]). Accordingly, a double stranded nucleic acid can interact by Watson-Crick base pairing to the adapter nucleic acid by strand invasion; for example, when the adaptor segment is a PNA.

Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, second paragraph because claim 4 is definite and clear.

II. 35 U.S.C. §103(a)

A. *Chan in view of Rubnitz*

Claims 1, 2, 4, 8-13, 15-21, and 23-26 stand rejected under 35 U.S.C. §103(a) as anticipated by Chan, et al. (J. Biol. Chem. (April 1999) 274: 11541-11548) ("Chan") in view of Rubnitz, et al., (Mol. Cell. Biol. (1984) 4:2253-2258) ("Rubnitz"). The examiner contends that an ordinary practitioner would wish to solve the problem that short fragments were inefficient substrates for recombination, recognized by Chan, by applying the teaching of Rubnitz.

Applicants respectfully traverse this rejection.

The examiner contends that "Chan was aware that short fragments were inefficient substrates for recombination (see page 11541, column 2)." Chan cites Rubnitz (having a publication date of 1984) for this proposition. However, Chan and other publications it cites (both published in 1996, *i.e.*, after Rubnitz) contradict these results on page 11547, col. 2, third paragraph (emphasis added), by citing that short nucleic acid constructs can mediate directed sequence changes:

> These results [*i.e.*, the *in vitro* results of Chan that show that sequence alteration of sup F gene in the SV40 vector can be mediated by short donor fragments alone] are somewhat consistent with other reports that short nucleic acid constructs can mediate directed sequence changes (10, 16).

The references cited by Chan to support the finding that short nucleic acid constructs can mediate directed sequence changes, *e.g.*, footnotes 10 and 16, were both published after Rubnitz. Accordingly, Chan recognized that short nucleic acid constructs can mediate directed sequence changes, and did not consider that the use of short donor nucleic acid was a problem for *in vitro* recombination. Therefore, there would be no motivation to combine Chan with Rubnitz to arrive at

the presently claimed invention because Chan did not consider the use of a short donor nucleic acid as a problem for *in vitro* recombination.

Furthermore, even if one skilled in the art understood Chan to convey the purported problem of a low level of homologous recombination with short fragments in an individual recombinant system, Chan achieves recombination efficiency, and overcomes the alleged low level of homologous recombination of short fragments in a linear system, by implementing a tri-component recombinant system having a TFO, donor linker, and short fragment donor nucleic acid.

A person having ordinary skill in the art would not be motivated to look to Rubnitz to increase the percent of homologous recombination because Chan solves this purported problem in the art by the disclosed means, e.g., the tri-component system. Even if a person having skill in the art were to look to Rubnitz based upon Chan's citation, Rubnitz does not solve the problem disclosed in Chan, e.g., low percent homologous recombination, by merely increasing the length of DNA. Rubnitz makes no mention of a tri-component system, or the fact that longer DNA segments always increase the percent of homologous recombination in every instance.

In addition, such a combination of the references is hindsight reasoning in view of the presently claimed invention where the addition of long fragments to a TFO-covalently attached to an adapter enhances the ability to replace nucleotides to any target site (¶0081). Replacement of nucleotides according to the presently claimed invention is not limited to the vicinity of the triple helix site as is the case in Chan (¶0081). Accordingly, Chan and Rubnitz do not render claims 1, 2, 4, 8-13, 15-21, and 23-26 obvious, and applicants respectfully request withdrawal of this rejection.

B. *Chan in view of Erdeniz*

Claims 1-5, 8-13, 15-21, and 23-26 stand rejected under 35 U.S.C. §103(a) as unpatentable over Chan in view of Erdeniz, et al., 7 Genome Research 1774-1183 (1997) ("Erdeniz").

Applicants respectfully traverse this rejection.

Applicants reiterate that Chan in view of Rubnitz do not render the claimed invention obvious, and further, that the addition of Erdeniz to these references is not sufficient to establish obviousness. There is no objective reason to combine the teachings of the references. Erdeniz discloses a method of allele amplification by polymerase chain reaction (PCR) using adaptamers (page 1181), and does not disclose TFO mediated gene correction or mutation. Modification of Chan and Rubnitz in view of Erdeniz to meet the claimed invention, because it was within the skill of the art to synthesize alleles by PCR, is not sufficient to provide motivation to make the claimed invention. Instead, there must be some objective reason to combine the teachings of the references.

Chan discloses a TFO system for provoking site-directed genome modification. Chan does not suggest a recombination system that uses long donor DNA fragments, nor does Chan disclose the ability to replace nucleotides at any target site. The Rubnitz and Erdeniz references are not concerned with TFO systems for genome modification. Rubnitz merely describes recombination frequency not involving a triple helix forming oligonucleotides. The Erdeniz reference teaches PCR-based allele replacement methods. Neither Rubnitz nor Erdeniz suggest carrying out homologous recombination based upon a system as in claim 1 involving introducing into a cell a nucleic acid targeting system comprising a third strand oligonucleotide, a donor nucleic acid, and an adapter segment, allowing the third strand oligonucleotide to bind to the native nucleic acid, and allowing homologous recombination to occur between the native and donor nucleic acid segments, wherein the donor nucleic acid is between more than 100 and 1,000,000 bases in length.

The claimed method allows replacement of nucleotides at any target site, even those hundreds of base pairs away from the triplex site (¶0081). The only suggestion for the examiner's combination of the isolated teachings of these references improperly stems from applicants' disclosure and not from the prior art. Accordingly, there is no objectively reasonable basis for combining these references, and the rejection of claims 1-5, 8-13, 15-21, and 23-26 under 35 U.S.C. §103(a) should be withdrawn.

C. *Chan in view of Rubnitz and further in view of Sato*

Claims 1, 2, 4, 8-21, and 23-26 stand rejected as unpatentable over Chan in view of Rubnitz, and further in view of U.S. Patent No. 5,770,408 to Sato, et al. ("Sato").

Applicants respectfully traverse this rejection.

Chan in view of Rubnitz, and further in view of Sato, do not render the claimed invention obvious. There is no objective reason to combine the teachings of the references, outside of applicants' disclosure. As noted above, Chan discloses a TFO-system for effecting DNA repair, and Rubnitz discloses the frequency of recombination in systems not involving triple helix forming oligonucleotides. Sato teaches a method for amplification of a base sequence by ligase chain reaction (LCR) wherein the method of amplification may have a linkage portion comprising hexaethyleneglycol. Neither Rubnitz nor Sato suggest carrying out homologous recombination based upon a system as in claim 1 for the reasons pointed out above. Furthermore, even though Sato discloses a hexaethyleneglycol linker, the combined references do not suggest the method of claims 11 and 14, dependent from claim 1, "wherein the adapter is linked to the third strand oligonucleotide through a spacer" (claim 11), and "wherein the spacer is a hexaethyleneglycol chain" (claim 14).

The only suggestion for the alleged combination of the isolated teachings of these references improperly stems from applicants' disclosure and not from the prior art. Accordingly, there is no objectively reasonable basis for combining these references, and the rejection of claims 1, 2, 4, 8-21, and 23-26 under 35 U.S.C. §103(a) should be withdrawn.

III.35 U.S.C. §112, first paragraph

Claims 1-21 and 23-26 stand rejected under 35 U.S.C. §112, first paragraph. The examiner contends that the claims are not enabled for *in vivo* methods of site directed mutagenesis of a target DNA molecule.

Applicants respectfully traverse this rejection.

First, applicants respectfully point out that the *ex vivo* methods disclosed in the specification correlate to *in vivo* methods of effecting homologous recombination and therefore, the claims enable all methods, *i.e.*, *ex vivo* and *in vivo*. This correlation is supported by Vasquez, et al. 2000, SCIENCE 290:530 ("Vasquez"). Vasquez shows that TFOs can induce mutations at the target genomic sites in somatic cells of adult mice; accordingly, gene targeting via triple helix formation is possible *in vivo*. Therefore, the rejection should be withdrawn.

Second, to expedite prosecution of the instant application, applicants have amended claims 1 and 23 to claim *ex vivo* methods. Support for the amendments appears throughout the specification, and is exemplified in at least Example 6. Accordingly, the rejection of claims 1-21 and 22-26 under Section 112, first paragraph for lack of enablement should be withdrawn because indeed, as the examiner has stated, the instant specification enables *ex vivo* methods (see Office Action, p. 8, No. 8).

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

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Respectfully submitted,

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